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Cellulose-decomposing microorganisms of light chestnut soils of the Volgograd region

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Abstract. The results of the dependence of the activity of cellulose-decomposing microorganisms on the anthropogenic impact on the soil are presented in the article. The qualitative reaction of microbial community to cellulose was assessed by the decomposition of linen fabric. The decomposition of the textile fibers and the increase of the weight of pieces of linen fabric by 30.64% in samples which were laid at the level of 10-20 cm in favourable soil conditions were demonstrated. It was determined that the highest microbial number was observed when a soil extract was cultivated on the Chapek medium, regardless of the soil conditions in which the microorganisms lived. The soils of the Volgograd region in the sphere of anthropogenic impact are contaminated and poor in micro- and macroelements, so they are not suitable for the life of the soil microbiota. That fact is evidenced by the lack of growth of microorganisms on an agarized soil starvation medium.

1. Introduction

Soil resistance to anthropogenic pollution is known to be in dependence on its physicochemical and agrobiological properties. Technogenic pollutants of organic nature are transformed in the soil with the participation of microorganisms [1-3].

Formation of light chestnut soils is a distinguish feature of the Volgograd region. At the same time, the composition of microbial species in the soil microbiota is mostly determined by the climatic conditions in the region and the technogenic impact. The processes of transformation of organic substances into humus and the processes of mineralization of organic matter to final products (such as CO₂ and H₂O) are important for maintaining the soil-plant integrity, as well as for maintaining the geological circulation of various chemical elements [4-6]. Soil microorganisms in their activities provide the uptake of mineral nutrients for plants, so they influence on plant growth and development [7]. Nitrogen-fixing and cellulose-decomposing microorganisms play an important role in replenishing of nutrients in the soil and maintaining soil fertility at the proper level. Analysis of data on the quantitative ratio of cellulose-decomposing microorganisms in the soil in various environmental conditions will allow identifying the significance of those biotic agents in the processes of soil formation in the conditions of the sharply continental climate of the Volgograd region.

Objective of the research is the identification of the dependence of the activity of cellulose-decomposing microorganisms on the anthropogenic impact on the soil.



2. Materials and methods

Researches were carried out on the territory of the Soviet district of Volgograd. As analysed plots, we used fields with favourable (plot No. 1) and unfavourable (plot No. 2) environment for cellulose-decomposing microorganisms. We used the following criteria to select a favourable plot: moisture, fertilization, soil tillage. The type and structure of the soils in the plots were the same.

The main method of determining the qualitative reaction of soil microbiota for cellulose was the method of decomposition of pieces of linen fabric. Three samples of linen fabric were laid on the studied areas in the soil at a depth of 0-10, 10-20 and 20-30 cm. The distance between the samples was 1 m. These samples were cut pieces of linen fabric with size 4×4 cm; they were attached to plates made of fiberglass. Fiberglass was chosen because, in turn, it was not susceptible to rapid decomposition as compared to linen fabric. The weight of linen cloths was 0.3234 g. At the same time, soil samples for microbiological examination were taken from a depth of 0-10 cm [8].

We assessed the qualitative reaction to cellulose in 1 month after the day when we put linen fabric into a soil. We noted the change of the weight of the pieces of linen fabric and visually inspected the decomposed tissue.

Soil samples with weight 1 g were taken from the named plots and packed in sterile laboratory glassware. They were delivered to the laboratory on the same day when the samples were selected. Samples store as allowed for no more than 24 hours at a temperature of 4-5°C.

The concentration of microbial cells in the samples was reduced to 10^7 by the dilution method. The samples were diluted in sterile physiological saline in the ratio 1:10. Bacterial suspension with volume 0.1 ml was sown on Petri dishes with agarized nutrient media. We used the following media: medium with meat hydrolyzate (MPA), medium of Chapek, and agarized soil starvation medium. Each sample contains three replicates. Next, the samples were incubated at room temperature for a week.

The first time we assessed the cultures of bacteria on agarized media in 1 day from the beginning of cultivation, and cultural properties were described during the germination of microorganisms on agarized nutrient media. The number of colony forming units (CFU) was counted using a ColonyStar device (microorganism colony counter). The morphological properties of bacteria were studied by preparing smears and staining by Gram.

3. Results and discussion

The method of "linen fabric", which was used in the work, is affordable, inexpensive, and reliable. According to the results it was possible to determine the qualitative reaction and the rate of decomposition of cellulose. Despite the presence of many modern methods, this method allowed the solving an environmentally significant problem of monitoring of the soil resource.

A visual inspection of pieces of linen fabric before the incubation in the soil and in 1 month after the laying in the soil allowed revealing the presence of tissue decomposition in the samples which were laid at a level of 10–20 cm in favourable soil conditions. Under unfavourable soil conditions (technogenic impact), no decomposition of flax was recorded (figure 1).

Gravimetric analysis of pieces of linen fabric before laying and in 1 month after laying in favourable soil conditions allowed establishing a decrease in the weight of pieces of linen fabric in three replications by an average of 30.64% as compared to initial weight. The decrease of weight of linen tissues indicated the activity of cellulose-decomposing microorganisms (table 1).

Table 1. Weight of pieces of linen fabric before laying and in 1 month after laying at a level of 10–20 cm in a soil with favourable conditions.

	Initial weight, g	Weight in 1 month after laying in a soil, g	%
sample 1	0.3234	0.2860	11.56
sample 2	0.3234	0.1953	39.61
sample 3	0.3234	0.1916	40.75
Average weight	0.3234	0.2243	30.64

The results indicate a high activity of the soil microbiota, which is a mediator between the soil and plants. The activity of the microbial community contributes to the accumulation of nutrients in the soil for the better growth of plants. Activity of soil microbial destructors generally maintains the soil-plant integrity.

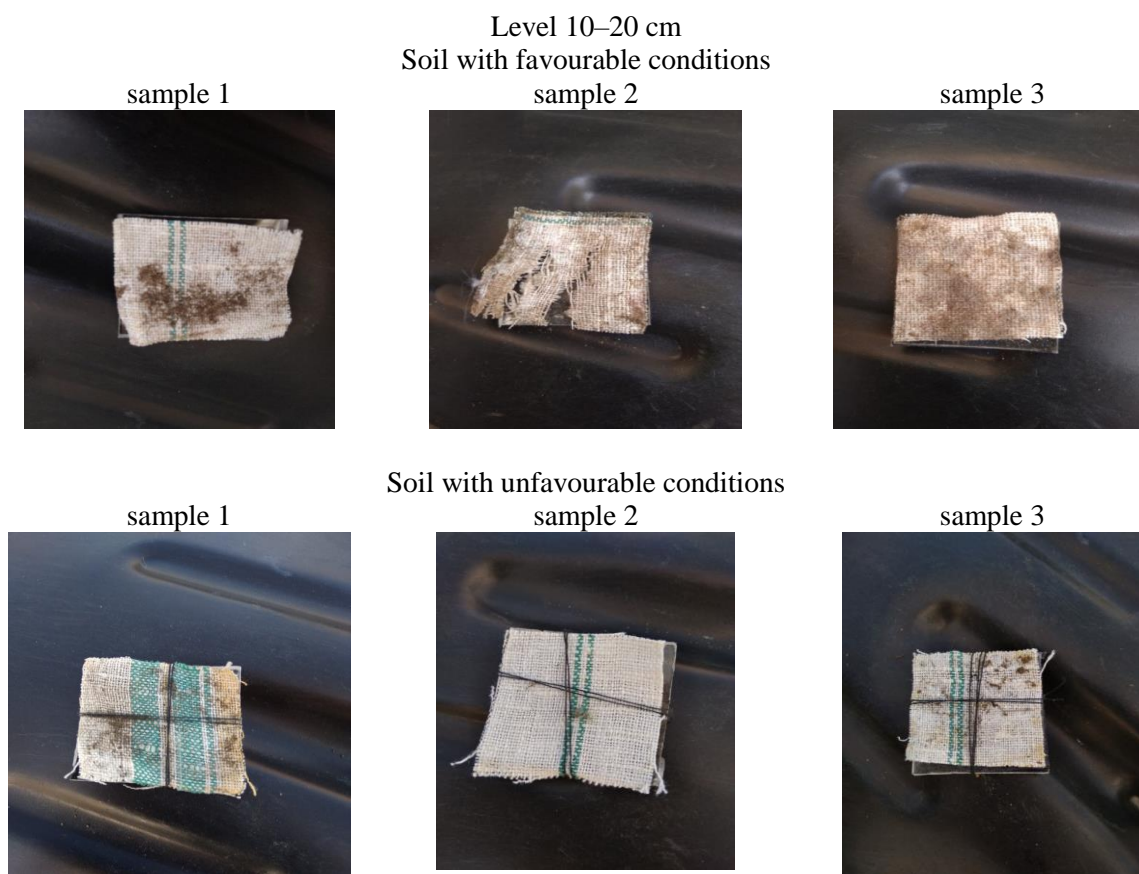


Figure 1. Results of visual analysis of destruction of pieces of linen fabric by cellulose-decomposing microorganisms in the soil.

According to our research, the dependence of microbial biomass formation on soil conditions was determined (table 2). So, in favourable environment, the largest total microbial number ($3.6 \cdot 10^{11}$) was demonstrated when a soil extract was grown on the Chapek medium, regardless of the conditions in which the microorganisms lived before. This fact indicates that microorganisms preference soils with pH = 4.8. Only microorganisms which were taken from soil with favourable conditions ($6.45 \cdot 10^9$) grew on agarized soil starvation medium. At the same time there was no growth of microorganisms which were taken from the soil with unfavourable conditions. The growth of microorganisms on the medium with meat hydrolysate (MPA) was also revealed. In a soil with favourable conditions, the microbial number was $2.25 \cdot 10^9$, which is significantly higher than the same indicator in the soil with unfavourable conditions.

Table 2. The total microbial number of cellulose-decomposing microorganisms in soil samples.

Nutrient medium	Soil with favourable conditions	Soil with unfavourable conditions
Agarized soil starvation medium	$6.45 \cdot 10^9$	No growth
Chapek medium	$3.60 \cdot 10^{11}$	$3.0 \cdot 10^6$
Medium with meat hydrolysate (MPA)	$2.25 \cdot 10^9$	$3.0 \cdot 10^4$

4. Conclusion

At present, Volgograd is one of the cities with a developed industrial sector. Industrial production has an impact on various areas of the environment, including the soil. Light chestnut soils with an alkaline environment prevail in the Volgograd region. The number of soil microbiota depends on the favourable conditions in the soil, namely: soil moisture, soil loosening, and temperature. That fact is significant for the Volgograd region, since its climate is characterized by sharply continental conditions, such as temperature changes, low water regime, weathering of the soil.

The activity of microorganisms detected in samples which were taken from soil with favourable conditions indicates that even in arid climatic conditions in the soils of Volgograd, soil microorganisms are able to actively decompose cellulose. It was noted that in the upper layers (10-20 cm) the presence of plant residues enhances the activity of cellulose-depleting microorganisms.

In light chestnut soils, that are sufficiently poor in nutrients for plants, the presence of cellulose-decomposing and nitrogen-fixing bacteria predetermines the fertility of those soils.

The results of the study of soil microbiota allowed to establish that the highest microbial number was during cultivation of soil suspension in the Chapek medium. This fact indicates that microorganisms prefer acidic soil. However, light chestnut soils are alkaline. The growth of microorganisms in an acidic environment can be explained by the fact that the mineral fertilizers applied to the soil, as well as the root excretions of plants into the rhizosphere, reduce the pH of the medium. That acidification makes favourable conditions for the soil microbiota.

On the contrary, the soils in the area of technogenic impact are contaminated and poor in micro- and macroelements. Plants are under technogenic stress, while their energy metabolism is disturbed, so the absorption of nutrient cations and the excretion of H^+ decreases. Such soils are not suitable for the life of the soil microbiota, which is confirmed by the lack of growth of microorganisms on the agarized soil starvation environment. The technogenic impact disrupts the processes of destruction of organic litter, and, consequently, the processes of soil formation.

The results of the study of the activity of cellulose-decomposing microorganisms should be used in the testing of mineral fertilizers on the effectiveness of their influence on soil microbial communities in the system "microorganism - soil - plant".

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